STANDARD OPERATING PROCEDURE

METHYLENE BLUE ACTIVE SUBSTANCES (MBAS)

Signature and Title

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U.S. ENVIRONMENTAL PROTECTION AGENCY
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DIVISION OF ENVIRONMENTAL SCIENCE AND ASSESSMENT
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1. SCOPE AND APPLICATION

1.1 This method is applicable to the measurement of methylene blue active substances (MBAS) in drinking water, surface water, domestic and industrial wastes. It is not applicable to measurement of surfactant-type materials in saline waters. They are calculated and reported in terms of the reference material, linear alkyl benzene sulfonate, LAS.

1.2 The reporting limit for this method is 0.1 mg/L. The upper limit of 2.0 mg/L can be extended by dilution of the sample.

1.3 This SOP is based on Standard Method 5540 C.

1.4 All analysts must satisfactorily perform a demonstration of capability (DOC) by meeting the method performance criteria in section 13.1 prior to performing sample analysis using this SOP.

2. SUMMARY OF METHOD

2.1 This test method is based upon the formation of a blue-coated chloroform extractable ion pair by the reaction of cationic methylene blue and an anionic surfactant.

2.2 The sample is mixed with an acidified, aqueous solution of methylene blue. Any resulting hydrophobic ion pair which may be formed is extracted successfully with chloroform. The combined chloroform extracts are washed with an acid solution to remove the less hydrophobic ion pairs that can be formed by potentially interfering substances. The chloroform layer retains the highly hydrophobic methylene blue-LAS ion pairs.

2.3 The intensity of the blue color remaining in the chloroform extract is measured photometrically at the wavelength of maximum absorption at 652 nm. This intensity is related to the concentration of LAS by means of a calibration curve or chart.

3. DEFINITIONS

See SOP # G-15 for definitions.

4. INTERFERENCES

4.1 Materials other than man made surface active agents which react with methylene blue are organically bound sulfates, sulfonates, carboxylate, phosphates, phenols, cyanates, thiocyanate and some inorganic ions such as nitrates and chlorides. However, the occurrence of these materials at interference levels is relatively rare and with the exception of chlorides may generally be disregarded.

4.2 Chlorides at concentration of 1000 mg/L shows a positive interference but the degree of interference has not been quantified. For this reason the method is not applicable to brine samples.

4.3 Presence of cationic surfactants and other cationic materials, such as, amines, are negative interferences.

4.4 Colored materials extractable into chloroform may interfere.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be kept to an absolute minimum by following the appropriate standard safety procedures, e.g. wear proper protective equipment, gloves, lab coat, and working inside hoods whenever possible. Refer to Edison Facility Safety Manual Region II, Part 2 - Laboratory Safety for specific guidelines.

5.2 The following chemicals have the potential to be highly toxic or hazardous, for detailed explanations consult the MSDS.

5.2.1 Chloroform.

5.2.2 Sulfuric Acid

6. APPARATUS AND MATERIALS

6.1 Spectrophotometer, for use at 652 nm, providing a light path of 1 cm or longer.

6.2 Separatory funnels - 500 mL, preferably with inert TFE stopcocks and stoppers.

7. REAGENTS AND SOLUTIONS

7.1 Stock LAS Calibration Standard, 1000 mg/L
Weigh an amount of LAS Reference Material equivalent to 1.00 g 100% active LAS. Actual activity is provided with each lot of reference material. Dissolve in deionized water and dilute to 1000 mL. Alternatively, use a commercially prepared stock solution.

7.2 Intermediate Standard, 10 mg/L
Dilute 10 mL of stock solution to 1000 mL with deionized water. Prepare fresh. Each time analysis is run.

7.3 Working Standards

The upper and lower value of the standard curve should not be modified. If a project requires a different range the upper and lower standards may be modified but the reporting limit must also be adjusted accordingly. Samples above the high standard (+10%) must be diluted. The mid-range standards listed below are recommended but may be modified by the analyst.

Dilute the intermediate standard with deionized water as follows:

Aliquot	Final Volume	Concentration
1.0 mL	100 mL	0.1 mg/L
2.0 mL	100 mL	0.2 mg/L
4.0 mL	100 mL	0.4 mg/L
10.0 mL	100 mL	1.0 mg/L
20.0 mL	100 mL	2.0 mg/L

7.4 Chloroform - $CHCl_3$ is toxic and is suspected of being a possible carcinogen - avoid ingestion or absorption through the skin.

7.5 Phenolphthalein Indicator Solution
Dissolve 80 mg phenolphthalein in 100 mL methanol.

7.6 Sodium Hydroxide, 1N
Dissolve 40 g sodium hydroxide in deionized water, cool, and dilute to 1000 mL. Store in a plastic container.

7.7 Sulfuric Acid, 1N
Slowly add 28 mL concentrated sulfuric acid to about 800 mL deionized water with constant mixing. Cool and dilute to 1000 mL with deionized water.

7.8 Methylene Blue Solution, 0.1%
Dissolve 100 mg methylene blue in 100 mL water.

7.9 Methylene Blue Reagent
Transfer 30 mL of 0.1% of methylene blue solution 1 L volumetric flask and add 500 mL of water. Add carefully 6.8 mL conc. H_2SO_4, and 50 g of sodium phosphate, monobasic, monohydrate, $NaH_2PO_4.H_2O$. Shake until dissolved. Dilute to 1000 mL with deionized water.

7.10 Wash Solution
Slowly add 6.8 mL conc. H_2SO_4 to 500 mL deionized water with constant mixing. Add 50 g $NaH_2PO_4.H_2O$ and shake until dissolved. Dilute to 1000 mL with deionized water.

7.11 Glass Wool - pre extract with $CHCl_3$ to remove interferences.

8. SAMPLE COLLECTION, PRESERVATION, STORAGE AND HOLDING TIMES

8.1 Samples are to be collected in plastic containers and refrigerated to 4°C.

8.2 The holding time for MBAS is 48 hours.

9. SAMPLE PREPARATION

9.1 Screen samples for chloride using the chloride electrode. Samples with more than 1000 mg/L chloride must be diluted before analysis to contain less than 1000 mg/L. Record chloride result and dilution required.

9.2 Place 100 mL of each standard in a separatory funnel.

9.3 Add 2 or 3 drops of phenolphthalein indicator.

9.4 Add 1N sodium hydroxide dropwise until the solution is pink.

9.5 Add 1N sulfuric acid dropwise until the pink color disappears.

9.6 Add 25 mL methylene blue reagent and mix.

9.7 Add 10 mL chloroform.

9.8 Rock the funnel vigorously for 30 seconds and then let the phases separate. Excessive agitation may cause the formation of an emulsion. Persistent emulsions may be broken by adding a few mLs (10 mL) isopropanol.

9.9 Swirl gently and allow to settle.

9.10 Draw off the chloroform into a second separatory funnel. Rinse the delivery tube of the first funnel with a small amount of chloroform.

9.11 Repeat steps 9.7 through 9.10 two more times. Combine the extracts in the second separatory funnel. If the blue color in the aqueous phase disappears, discard the sample and reanalyze a smaller aliquot.

9.12 Add 50 mL wash solution to the second funnel and shake vigorously for 30 seconds. Emulsions should not form at this point.

9.13 Let the phases separate, swirl gently, and drain the chloroform through glass wool into a 100-mL volumetric flask.

9.14 Extract the wash solution twice with 10 mL chloroform and add to the flask.

9.15 Rinse the glass wool into the flask with chloroform and dilute to volume.

10. INSTRUMENT OPERATING CONDITIONS

10.1 Turn on the spectrophotometer and the PC. Open the HachLink 2000 software. Open the MBAStemp file in Excel. An empty window in tabled format should open.

10.2 From the main menu on the spectrophotometer, press the softkey under single wavelength. Press goto wavelength. Enter 652 and enter.

11. SAMPLE ANALYSIS

11.1 Pour an aliquot of the extracted standard or sample into a 16 X 100 culture tube.

11.2 Place the 0 standard into the spectrophotometer.

11.3 Press the print key to send the readings to Hachlink. If sample information does not appear on the computer screen, check the connections and software settings.

11.4 Place the next standard into the sample cell and cover.

11.5 Repeat step 11.4 with the remaining standards. Double click on the Hachlink standards data. The software will automatically open a second Excel spreadsheet (Book 1) and enter the standards information from Hachlink.

11.6 Highlight, copy and paste the header and standards data into the MBAS template.

11.7 Correct the true value of the standards and verify that the correlation coefficient is >0.995.

11.8 Prepare and analyze the AQC's and samples as above repeating the process until all of the samples and associated QC are analyzed.

11.9 After the analysis is complete, go to the Hachlink generated Excel spreadsheet. Highlight, copy and paste all of the rows with the sample information from the data column on the left to the units column.

11.10 Return to the MBAS template and paste the information in the sample section of the spreadsheet.

11.11 Enter any sample dilutions manually and enter the full sample ID's into the MBAS template. Rename the spreadsheet in the format of MM/DD/YYMBAS.

11.12 Repeat this process until all of the samples and associated QC are analyzed. If any of the samples have a higher absorbency than the highest standard in the calibration

curve, dilute the sample with chloroform and reanalyze.

12. DATA ANALYSIS AND CALCULATIONS

All calculations are performed in the Excel spreadsheet. A linear regression is used to calculate sample results in mg/L by using the absorbances of samples and standards. Multiplication for sample dilutions and the subtraction of the color/turbidity blank results must be done manually in Excel.

13. METHOD PERFORMANCE

A demonstration of capability (DOC) should be performed each time there is a significant change in the chemistry of the method, a major modification to an existing instrument, or a new instrument is installed. A DOC is performed by each analyst designated to analyze samples using this method. An annual check must subsequently be performed and documented for each analyst using this method.

13.1 Accuracy and Precision

13.1.1 Demonstration of Capability

A demonstration of capability study must be performed and documented for each analyst using this method. The study should consist of the analysis of four standards which are from a source independent of the standard curve. The results of the standards must be within the acceptance criteria supplied by the manufacturer or within 20% if none are specified. The % RSD should be within 20%. The results of the accuracy and precision study (true value, % recovery, standard deviation and % RSD) are maintained by the Quality Assurance Officer for each analyst and are located in the Central Branch File.

13.1.2 Continuing Demonstration of Capability

An annual continuing demonstration of capability study must be performed and documented. It may consist of either successfully analyzing a PT sample or analyzing 2 sets of AQC standards to within control limits as stated in section 13.1.1. The results of the continuing accuracy and precision study (true value, % recovery, standard deviation and % RSD or final report from the PT provider) are maintained by the Quality Assurance Officer for each analyst and are located in the Central Branch File.

13.2 Limit of Quantitation (LOQ)

The Laboratory performs a Limit of Quantitation (LOQ) study on an annual basis for analytes associated with chemistry methods. The validity of LOQ is confirmed by successful analysis of a Laboratory Fortified Blank (LFB) at approximately 2X the reporting limit. The acceptance criteria for each analyte is $\pm$ 30% of the true value. After this study is completed, it is reviewed and approved by the Laboratory Management. A summary of all LOQ study performance is maintained in the Laboratory's Central File.

14. QUALITY CONTROL

14.1 Calibration Curve

Acceptance Criteria - A minimum of 5 standards and a blank must be used to generate the calibration curve. The correlation coefficient must be $\geq$0.995.

Corrective Action - If the correlation coefficient of the calibration curve, consisting of at least five standards and a blank, is <0.995, the calibration is disallowed. The analysis must be terminated, and repeated after correcting the problem.

14.2 Instrument Performance Check (IPC) Standard or Initial Calibration Verification (ICV) and Continuing Calibration Verification (CCV) Standard.

Acceptance Criteria - Analyze the IPC solution for all determinations immediately following each calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution immediately following calibration must verify that the instrument is within $\pm$10% of the true value. Subsequent analyses of the continuing IPC solution must be within $\pm$10% of the true value.

Corrective Action - If the calibration cannot be verified within the specified limits, analysis must be discontinued, the cause determined and/or in the case of drift the instrument re-calibrated. All samples following the last acceptable IPC solution must be reanalyzed.

14.3 Laboratory Reagent Blank (LRB),Prep Blank (PB), Initial Calibration Blank (ICB) and Continuing Calibration Blank (CCB)

Acceptance Criteria - Analyze a blank along with each batch of 20 or fewer samples. All LRB/PB/ICB/CCB results must be < the Reporting Limit.

Corrective Action - If the results of the LRB/PB/ICB/CCB are > the Reporting Limit then all associated samples with a concentration of ≤10x the amount found in the LRB/PB/ICB/CCB should be reprepared and reanalyzed (sample results ≥10x the amount found in the LRB/PB/ICB/CCB are not considered to be affected by the blank contamination or drift).

If the samples cannot be reprepared, then all affected sample results must be either 1) qualified accordingly, or 2) the reporting limit is raised to the amount found in the blank. Check with the team leader/section chief to determine which option should be used.

14.4 Laboratory Fortified Blank (LFB), Analytical Quality Control Samples (AQC's) or Quality Control Samples (QCS)

Acceptance Criteria - Analyze two LFB/AQC/QCS samples with each batch of 20 or fewer samples. Calculate accuracy as percent recovery using the following equation:

$$\% \text{ Recovery} = \frac{\text{LFB/AQC/QCS}}{\text{s}} \times 100$$

where:

LFB/AQC/QCS = control sample results determined by laboratory

s = concentration equivalent of analyte added to fortify the LFB/AQC/QCS solution.

The % recovery of the LFB/AQC/QCS should be within 85-115%. The relative percent difference (RPD) of the duplicates should not exceed 20% for aqueous standards.

Corrective Action - If the % recovery or RPD results are outside the required control limits, the affected samples should be reprepared and reanalyzed. If the

samples cannot be reprepared, then all affected sample results must be qualified accordingly.

14.5 Laboratory Fortified Matrix(LFM) or Matrix Spike(MS) Recovery

Acceptance Criteria - Add a known amount of the target analyte to a minimum of one sample per batch of 20 or less samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. Calculate the percent recovery, corrected for background concentration measured in the unfortified sample aliquot as per the equation below. The % recovery should be 80-120%.

$$R = \frac{Cs - C}{s} \times 100$$

where:
- R = percent recovery,
- Cs = fortified sample concentration,
- C = sample background concentration, and
- s = conc. equivalent of spike added to sample.

Corrective Action - If the % recovery of the LFM/MS is outside the required control limits, and the laboratory performance is shown to be in control, the recovery problem encountered is judged to be matrix related, not system related. The native sample result of the sample used to produce the LFM/MS must be qualified accordingly.

Note: The % recovery of the LFM/MS is not evaluated if the result of the unfortified sample concentration is ≥1X the level used to fortify the sample.

15. REPORTING AND VALIDATION

15.1 Reporting Limits

The reporting limits are calculated based on the concentration of the lowest calibration standard analyzed. The reporting limits are matrix and dilution dependent. All results are reported to 2 significant figures.

15.2 Sample Data Package

The sample data package should include the following items, where applicable.

The analyst may also include other information that may be pertinent to the analysis such as project narratives, etc.

1. Sample preparation information
2. Sample analysis data
3. Instrument calibration data
4. Instrument/computer printouts
5. Data summary checklist with all relevant information entered

15.3 Laboratory Information Management System (LIMS)

The analyst enters the data on the LIMS under the appropriate analytical codes.

15.4 Data Validation

The data package is given to the reviewer. The review is done by a peer who was not involved in the analysis. Upon completion of the review, including validation of all the appropriate codes in the LIMS for the particular project(s), the data reviewer will sign and date the QA/QC Checklist.

15.5 Data Records

A copy of each analytical data package is made for each project in the package and placed in the bin identified for the designated project file. The records for this designated project file are filed in our locked record cabinets once all data from the project, e.g., non-metal inorganic data, organic data, microbiology data, etc. has been reviewed by the appropriate staff.

16. POLLUTION PREVENTION

16.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the USEPA recommends recycling as the next best option.

16.2 The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes

should reflect anticipated usage and reagent stability.

16.3 For information about pollution prevention that may be applicable to laboratories, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's Department of Government Regulations and Science Policy, 115 16th Street N.W., Washington D.C 20036, (202)872-4477.

17. WASTE MANAGEMENT

The USEPA requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any water discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the Region 2 SOP G-6, "Disposal of samples and hazardous wastes in Regional Laboratory".

18. REFERENCES

18.1 Standard Methods for the Examination of Water and Wastewater, Method 5540 C., 20th Edition, 1998

18.2 Annual Book of ASTM Standards, Vol. 11.02 Water (11) p. 75 (1988).